Research paper

Synthesis and photophysical characterization of luminescent lanthanide complexes of nucleotide-functionalized cyclen- and dipicolinate-based ligands

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ABSTRACT

Luminescent Eu(III)- and Tb(III)-complexes based on functionalized tetraazamacrocycle (cyclen) or dipicolinic acid (dpa) metal binding sites, and carrying 2′- or 5′-linked uridine moieties were prepared. The light-harvesting antennae were either a coumarin (in the cyclen-based architectures) or the dpa-moiety itself. Antenna excitation resulted in metal-centered emission for all complexes. The presence of the uridine resulted in less intense lanthanide emission compared to non-nucleotide-modified reference compounds. Nd(III)-complexes of cyclen ligands carrying a uridine but without a sensitizing antenna were also synthesized; these are envisioned as energy transfer acceptors to the Eu(III)-complexes. The possibility for Eu-to-Nd energy transfer was probed. The reported complexes are models for oligonucleotide-attached lanthanide probes.

1. Introduction

The study of nucleic acid structure and function is central to areas ranging from fundamental molecular biology to cancer diagnostics and therapy [1–3]. Chemical probes that facilitate such investigations continue to be in high demand. Metal complexes are some of the most important such probes due to their structural versatility, synthetic tractability, and tunable reactivity [4–11]. Metal ions can provide spectroscopic handles that are not available in the native structure. EPR-active, NMR shift-modulating, and luminescent centers have all been successfully incorporated into oligo- and polynucleotides [5,12].

Lanthanide(III)-ions (Ln(III)) have played important roles in this area in large part due to their unique narrow emission bands and long-lived luminescence arising from forbidden f-f transitions [13]. For example, the binding of chiral Ln(III)-complexes to nucleic acids can be followed using a range of techniques including luminescence spectroscopy; in some cases sequence-selective binding was achieved [14,15]. Ln(III)-ions have ionic radii comparable to Ca^{2+}, which is an essential, but spectroscopically silent determinant of DNA- and RNA-structure. Replacement of Ca^{2+} and Mg^{2+} with Ln^{3+} imparts attractive luminescent properties onto the nucleic acid. The Ln(III)-emission can be observed upon laser excitation. Alternatively, nearby nucleobases can harvest the excitation light and can sensitize Ln(III)-luminescence. This so-called antenna effect is often relied on to overcome the inherently low Ln(III)-absorptions [16]. Ln(III)-luminescence provides information on the metal ion's coordination environment including types of donor atoms, number of individual metal ion binding sites, number of metal-bound water molecules, and the distance between the metal ions [12]. Ln(III)-luminescence has also been harnessed for sensitive nucleic acid detection. Such experiments often rely on bringing together the Ln(III)-ion and a sensitizing chromophore on the target nucleic acid template. Sequence-selectivity is provided by base-pairing between the probe strands and the template [17–19]. Apart from luminescence properties, magnetic properties of lanthanides are also useful for nucleic acid detection. Gd(III)-contrast agents for the MRI-detection of intracellular DNA [20], as well as Nd(III) PARACEST-agents interacting with DNA and other phosphate esters have been developed [21]. Finally, the hard Lewis acidity of Ln(III)-ions, along with that of Ce(IV), has enabled the construction of efficient small-molecule [22], oligonucleotide- [23], and oligopeptide-based [24] nuclease mimics [25].

Catalytic nucleic acids are responsible for ribosomal protein synthesis, and are thus essential to life on Earth [26]. RNA and DNA sequences catalyzing transformations as varied as amide- and phosphate ester hydrolysis and Diels-Alder reactions are known [27–29]. Nucleic acid catalysts have numerous advantages over protein-based enzymes, including straightforward

identification of active sequences, reproducible chemical synthesis, and robustness. However, in order for these catalysts to live up to their full potential, their mechanisms need to be studied. Thus, chemical tools that enable the *in situ* probing of subtle DNA and RNA conformational changes would be important. Luminescent Lns can participate in energy transfer as donors (D) to other Lns, e.g. Eu(III) or Tb(III) to Nd(III) [30], as well as to organic acceptors (A), e.g. Eu(III) to Cy3 [31] or Tb(III) to GFP [32]. The former is useful for investigating components ~10 Å or less apart, while the latter works over longer distances, up to ~100 Å [30,31]. As the energy transfer efficiency decreases sharply ($1/r^6$) with increasing D-A distance, small distance variations can be readily detected [31]. Here, we report the synthesis and photophysical characterization of a series of Ln(III)-complex model compounds of DNA-bound luminescent probes.

2. Experimental procedures

Commercially available chemicals were of reagent-grade purity or better and were used without purification unless otherwise noted. Compounds **1-Me** [33], **2b** [34], **9** [35,36], **11**, **13** [37] and **17** [38] were synthesized using previously published procedures.

Analytical thin-layer chromatography (TLC) was carried out on TLC plates precoated with silica gel 60 F_{254}. Visualization of TLC was accomplished using a UV lamp followed by charring with potassium permanganate stain (3 g $KMnO_4$, 20 g K_2CO_3, 5 mL 5% w/v aqueous NaOH, 300 mL H_2O). Flash chromatography was performed using silica gel 60. HPLC analyses were performed with an Agilent Technologies 1100 system using a Chromolith® Performance RP-18 end-capped 100×4.6 mm column. Analytical HPLC used a binary isocratic method (pump A: 0.1% TFA containing water, pump B: 0.1% TFA containing acetonitrile; 75% B for 10 min). The flow rate used for analytical column was 1 mL/min and detection was carried out with a photodiode array detector. Freeze drying was performed using a HETOSICC freeze dryer (HETO LAB Equipments). Centrifugation was performed using a centrifuge 5702 (Eppendorf) at 4400 rpm.

^1H and ^{13}C NMR spectra were obtained using an Agilent 400 (400 MHz and 101 MHz, respectively) spectrometer. Chemical shifts are reported relative to residual solvent signals unless otherwise noted ($CDCl_3$: ^1H: δ 7.26, ^{13}C: δ 77.16; CD_3OD: ^1H: δ 3.31, ^{13}C: δ 49.00; D_2O: ^1H: 4.64, ^{13}C: 48.00 for an internal standard of CD_3OD). NMR data are assumed to be first order, and the apparent multiplicity is reported as "s" = singlet, "d" = doublet, "dd" = doublet of a doublets, "t" = triplet, "q" = quartet, "m" = multiplet, or "brs" = broad singlet. Italicized elements are those that are responsible for the chemical shifts. High-resolution electrospray ionization mass spectra (HR-ESI-MS) were obtained on a Bruker MicroTOF ESI mass spectrometer, or by the mass spectrometry service of the Ecole Polytechnique Federale de Lausanne or at the Organisch Chemisches Institut WWU Münster. Low-resolution mass spectra were obtained on a LC–MS system (Agilent 1100 LC system and Waters MicromassZq ESci mass spectrometer). UV–vis spectra were obtained on Varian Cary 100 Bio UV–vis spectrophotometer. The emission and excitation spectra and lifetimes were measured on a Horiba FluoroMax-4P spectrophotometer.

2.1. Synthesis

3: Compound **1-Me** (200 mg, 1.1 mmol, 1 equiv) was dissolved in CH_2Cl_2/THF (7:4, 22 mL) and the solution was stirred for 5 min. To the stirring mixture were added 6-Cl-HOBt (380 mg, 2.2 mmol, 2 equiv), TEA (1.6 mL, 11 mmol, 10 equiv), EDCI (420 mg, 2.2 mmol, 2 equiv), and 2′-amino-2′-deoxyuridine (268 mg, 1.1 mmol, 1 equiv) consecutively. The reaction mixture was stirred

under Ar at ambient temperature for 24 h. The solvents were removed under reduced pressure and the resulting oil was purified with silica gel column chromatography (5% MeOH/CH_2Cl_2) to yield 253 mg (56%) of **3** as a white solid: ^1H NMR (400 MHz, CD_3OD, δ) 3.82 (dd, *J* = 12, 20 Hz, C*H*₂, 2H), 4.01 (s, C*H*₃, 3H), 4.16 (s, C*H*, 1H), 4.37–4.41 (m, C*H*, 1H), 4.74–4.80 (m, C*H*, 1H), 5.76 (d, *J* = 8 Hz, C*H*, 1H), 6.17 (d, *J* = 8 Hz, C*H*, 1H), 8.06–8.15 (m, C*H*, 2H), 8.22–8.27 (m, C*H*, 2H); ^{13}C NMR (101 MHz, CD_3OD, δ): 52.1 (*C*H₃), 55.8 (*C*H), 61.7 (*C*H₂), 71.0 (*C*H), 87.1 (*C*H), 87.3 (*C*H), 101.8 (*C*H), 125.2 (*C*H), 127.3 (*C*H), 138.9 (*C*H), 141.3 (*C*H), 146.6, 149.3, 151.2, 164.5, 164.7, 165.1; TLC R_f = 0.14 (5% MeOH/CH_2Cl_2); LR-ESI-MS (*m/z*) [M+H]⁺ calcd for $C_{17}H_{18}N_4O_8$, 407.1; found, 407.1; HR-ESI-MS obsd 429.1025, calcd 429.1017 [(M+Na)⁺, M = $C_{17}H_{18}N_4O_8$].

4: To compound **3** (50 mg, 0.12 mmol, 1 equiv) dissolved in THF/MeOH/H_2O (3:2:2, 2 mL) was added NaOH (25 mg, 0.62 mmol, 5 equiv) and the solution was stirred at ambient temperature for 14 h. The solvents were removed under reduced pressure and the resulting residue was dissolved in water (2 mL). To this solution Dowex resin (H⁺, activated, 200 mg) was added and swirled for 10 min. The resin was filtered off and the filtrate was dried under reduced pressure to yield 43 mg (90%) of **4** as a colorless solid: ^1H NMR (400 MHz, CD_3OD, δ) 3.77–3.88 (m, C*H*₂, 2H), 4.17 (s, C*H*, 1H), 4.39–4.42 (m, C*H*, 1H), 4.78–4.85 (m, C*H*, 1H), 5.76 (d, *J* = 8 Hz, C*H*, 1H), 6.20 (d, *J* = 8 Hz, C*H*, 1H), 8.07–8.17 (m, C*H*, 2H), 8.23–8.31 (m, C*H*, 2H); ^{13}C NMR (101 MHz, CD_3OD, δ): 55.9 (*C*H), 61.7 (*C*H₂), 71.0 (*C*H), 87.0 (*C*H), 87.1 (*C*H), 101.9 (*C*H), 125.3 (*C*H), 127.3 (*C*H), 139.2 (*C*H), 141.3 (*C*H), 146.8, 149.1, 151.2, 164.6, 166.0; LR-ESI-MS (*m/z*) [M+H]⁺ calcd for $C_{16}H_{16}N_4O_8$, 393.1; found, 393.1; HR-ESI-MS obsd 437.0677, calcd 437.0680 [(M−H+2Na)⁺, M = $C_{16}H_{16}N_4O_8$].

5: Compound **1-Me** (200 mg, 1.1 mmol, 1 equiv) was dissolved in THF (20 mL) and stirred for 5 min. To the stirring mixture were added 6-Cl-HOBt (380 mg, 2.2 mmol, 2 equiv), TEA (1.6 mL, 11 mmol, 10 equiv), EDCI (420 mg, 2.2 mmol, 2 equiv) and stirring was continued for another 5 min. To this mixture was added 5′-amino-5′-deoxyuridine (268 mg, 1.1 mmol, 1 equiv) dissolved in water (1 mL). The reaction mixture was stirred under Ar at ambient temperature for 24 h. The solvents were removed under reduced pressure and the resulting oil was purified with silica gel column chromatography (5% → 7.5% MeOH/CH_2Cl_2) to yield 101 mg (23%) of **5** as an off white solid: ^1H NMR (400 MHz, CD_3OD, δ) 3.72–3.88 (m, C*H*₂, 2H), 4.03 (s, C*H*₃, 3H), 4.06–4.11 (m, C*H*, 1H), 4.13–4.20 (m, C*H*, 2H), 5.64 (d, *J* = 8 Hz, C*H*, 1H), 5.82 (d, *J* = 4 Hz, C*H*, 1H), 7.70 (d, *J* = 8 Hz, C*H*, 1H) 8.11–8.17 (m, C*H*, 1H), 8.26–8.36 (m, C*H*, 2H); ^{13}C NMR (101 MHz, CD_3OD, δ): 40.7 (*C*H₂), 52.8 (*C*H₃), 70.9 (*C*H), 73.4 (*C*H), 82.2 (*C*H), 90.2 (*C*H), 101.8 (*C*H), 125.6 (*C*H), 127.7 (*C*H), 139.5 (*C*H), 141.7 (*C*H), 146.1, 149.5, 151.1, 165.1, 165.2, 165.8; TLC R_f = 0.17 (7.5% MeOH/CH_2Cl_2); LR-ESI-MS (*m/z*) [M+H]⁺ calcd for $C_{17}H_{18}N_4O_8$, 407.1197; found, 408.1; HR-ESI-MS obsd 429.1019, calcd 429.1017 [(M+Na)⁺, M = $C_{17}H_{18}N_4O_8$].

6: To compound **5** (30 mg, 0.074 mmol, 1 equiv) dissolved in H_2O (2 mL) was added NaOH (25 mg, 0.62 mmol, 8 equiv) and the solution was stirred at ambient temperature for 6 h. The solvents were removed under reduced pressure and the resulting residue was dissolved in water (2 mL). To this solution Dowex resin (H⁺, activated, 500 mg) was added and swirled for 10 min. The resin was filtered off and the filtrate was dried under reduced pressure to yield 30 mg (quantitative) of **6** as a colorless solid: ^1H NMR (400 MHz, CD_3OD, δ) 3.71–3.87 (m, C*H*₂, 2H), 4.09–4.21 (m, C*H*, 3H), 5.65 (d, *J* = 8 Hz, C*H*, 1H), 5.83 (d, *J* = 4 Hz, C*H*, 1H), 7.73 (d, *J* = 8 Hz, C*H*, 1H), 8.10–8.17 (m, C*H*, 1H), 8.26–8.32 (m, C*H*, 2H); ^{13}C NMR (101 MHz, CD_3OD, δ): 40.8 (*C*H₂), 71.0 (*C*H), 73.5 (*C*H), 82.7 (*C*H), 90.0 (*C*H), 101.6 (*C*H), 124.9 (*C*H), 127.0 (*C*H), 139.0 (*C*H), 141.4 (*C*H), 147.8, 149.4, 150.9, 164.6, 165.0, 166.8;

LR-ESI-MS (m/z) [M+H]$^+$ calcd for $C_{16}H_{16}N_4O_8$, 393.1; found, 393.1; HR-ESI-MS obsd 437.0674, calcd 437.0680 [(M−H+2Na)$^+$, M = $C_{16}H_{16}N_4O_8$].

7Ln: Dipicolinic acid (300 mg, 1.8 mmol, 1 equiv) was dissolved in water and the pH was adjusted to 8 with NaOH (1 M). Respective LnCl$_3$·6H$_2$O were dissolved in water (0.5 equiv, 1 mL). The dipicolinate solution was added dropwise to the stirring LnCl$_3$ solution while maintaining the pH at 7–8. The reaction mixture was stirred at ambient temperature for 16 h. The white precipitate formed was separated by centrifugation and used for next step.

General procedure for **L^{1}Ln** and **L^{2}Ln**: Lanthanide bisdipicolinate complex **7Ln** (0.122 mmol, 1 equiv) was dissolved in water (1 mL). Compound **4** or **6** (0.127 mmol, 1 equiv) was also dissolved in water and the pH was adjusted to 8. The two solutions were combined and another 1 mL of water was added. The reaction mixture was stirred at ambient temperature for 18 h. The reaction mixture was centrifuged and the supernatant was separated and freeze dried to yield a white solid (**L^{1}Eu**, 90% and **L^{2}Eu**, 76%, Tb, 54%): HR-ESI-MS (m/z) for **L^{1}Eu** [M+H]$^+$ calcd for $C_{30}H_{21}N_6O_{16}EuNa_2$, 921.0105; found, 921.0150. HR-ESI-MS (m/z) for **L^{2}Eu** [M+H]$^+$ calcd for $C_{30}H_{21}N_6O_{16}EuNa_2$, 921.0105; found, 921.0151. We could not obtain satisfactory HR-ESI-MS data for **L^{1}Tb**. HPLC chromatograms are shown in the Supporting Information.

General procedure for **8** and **16**: 2′-amino-2′-deoxyuridine or 5′-amino-5′-deoxyuridine (500 mg, 2.06 mmol, 1 equiv) was suspended in MeOH (15 mL). DMAP (251 mg, 2.06 mmol, 1 equiv) was added and stirring was continued for 5 min. To the stirring mixture was added chloroacetic anhydride (1.41 g, 8.23 mmol, 4 equiv) and the clear solution was stirred at ambient temperature for 4 h. The solvent was removed under reduced pressure and the resulting oil was purified by silica gel column chromatography (10% MeOH/CH$_2$Cl$_2$) to obtain 498 mg (76%) of **8** or 296 mg (45%) of **16** as a white solid. For **8**: ^{1}H NMR (400 MHz, D$_2$O, δ) 3.58–3.70 (m, CH$_2$, 2H), 3.93 (s, CH$_2$, 2H), 3.98–4.02 (m, CH, 1H), 4.16–4.20 (m, CH, 1H), 4.36–4.41 (m, CH, 1H), 5.73 (d, J = 8.0 Hz, CH, 1H), 5.86 (d, J = 8.0 Hz, CH, 1H), 7.71 (d, J = 8.0 Hz, CH, 1H); ^{13}C NMR (101 MHz, CD$_3$OD, δ): 41.8 (CH$_2$), 55.5 (CH), 61.2 (CH$_2$), 69.7 (CH), 86.2 (CH), 86.9 (CH), 102.6 (CH), 141.5 (CH), 151.5, 165.7, 169.8; TLC R_f = 0.25 (10% MeOH/CH$_2$Cl$_2$); LR-ESI-MS (m/z) [M+H]$^+$ calcd for $C_{11}H_{14}N_3O_6Cl$, 320.1; found, 320.1; HR-ESI-MS obsd 342.0483, calcd 342.0463 [(M+Na)$^+$, M = $C_{11}H_{14}ClN_3O_6$]. For **16**: ^{1}H NMR (400 MHz, D$_2$O, δ) 3.41–3.45 (m, CH$_2$, 2H), 3.92–3.95 (m, CH, 2H), 3.96 (s, CH$_2$, 2H), 4.15–4.19 (m, CH, 1H), 5.60 (d, J = 4.0 Hz, CH, 1H), 5.70 (d, J = 8.0 Hz, CH, 1H), 7.49 (d, J = 8.0 Hz, CH, 1H); ^{13}C NMR (101 MHz, CD$_3$OD, δ) 40.6 (CH$_2$), 42.0 (CH$_2$), 70.3 (CH), 72.9 (CH), 81.6 (CH), 90.5 (CH), 102.1 (CH), 142.1 (CH), 169.9; TLC R_f = 0.23 (10% MeOH/CH$_2$Cl$_2$); LR-ESI-MS (m/z): [M+H]$^+$ calcd for $C_{11}H_{14}N_3O_6Cl$, 320.1; found, 320.1; HR-ESI-MS obsd 342.0472, calcd 342.0463 [(M+Na)$^+$, M = $C_{11}H_{14}ClN_3O_6$].

10: compound **9** (200 mg, 0.5 mmol, 1 equiv), NaHCO$_3$ (84 mg, 1 mmol, 2 equiv), KI (83 mg, 0.5 mmol, 1 equiv) were suspended in DMF/acetonitrile (1:10, 22 mL) and stirred for 15 min at 45 °C. Compound **8** was dissolved in DMF (3 mL), the solution was diluted with acetonitrile (20 mL), and this solution was added to the stirring mixture dropwise over 2 h. The reaction mixture was stirred under Ar at 45 °C for 48 h. The reaction mixture was filtered and the filtrate was dried under reduced pressure. The resulting oil was purified with silica gel (neutralized by pre-treating with a mixture of NH$_4$OH/MeOH/CH$_2$Cl$_2$ (0.5:3:8)) column chromatography (2.5 → 5 → 10% MeOH/CH$_2$Cl$_2$) to yield 177 mg (51%) of **10** as a pale yellow solid: ^{1}H NMR (400 MHz, CD$_3$OD, δ) 1.46 (s, CH$_3$, 18H), 2.52–3.26 (m, CH$_2$, 18H), 3.34–3.51 (m, CH$_2$, 4H), 3.78 (brs, CH$_2$, 2H), 4.12 (s, CH, 1H), 4.26–4.35 (m, CH, 1H), 4.53–4.60 (m, CH, 1H), 5.74 (d, J = 8.0 Hz, CH, 1H), 6.04 (d, J = 8.0 Hz, CH, 1H), 8.06 (d, J = 8.0 Hz, CH, 1H); ^{13}C NMR (101 MHz, CD$_3$OD, δ) 27.1 (CH$_3$), 43.5 (CH$_2$), 45.5 (CH$_2$), 49.1 (CH$_2$), 49.9 (CH$_2$), 50.9 (CH$_2$),

54.2 (CH$_2$), 55.1 (CH$_2$), 55.7 (CH$_2$), 55.8 (CH), 55.9 (CH$_2$), 61.7 (CH$_2$), 70.9 (CH), 81.2, 87.0 (CH), 87.5 (CH), 101.7 (CH), 141.3 (CH), 151.3, 164.5, 171.4; TLC: R_f = 0.24 (10% MeOH/CH$_2$Cl$_2$); LR-ESI-MS (m/z) [M+H]$^+$ calcd for $C_{31}H_{53}N_7O_{10}$, 684.8; found, 684.2; HR-ESI-MS obsd 684.3915, calcd 684.3927 [(M+H)$^+$, M = $C_{31}H_{53}N_7O_{10}$].

12: Compound **10** (62 mg, 0.092 mmol, 1 equiv), Cs$_2$CO$_3$ (45 mg, 0.14 mmol, 1.5 equiv), and KI (23 mg, 0.14 mmol, 1.5 equiv) were suspended in acetonitrile (7 mL) and stirred for 15 min at 55 °C. Coumarin derivative **11** (27 mg, 0.092 mmol, 1 equiv) was dissolved in acetonitrile (1 mL) and was added to the stirring mixture dropwise over 30 min. The reaction mixture was stirred under Ar at 55 °C for 24 h. The reaction mixture was filtered and the filtrate was dried under reduced pressure. Resulting residue was purified with silica gel (neutralized by pre-treating with a mixture of NH$_4$OH/MeOH/CH$_2$Cl$_2$ (0.5:3:8)) column chromatography (2.5 → 5 → 10% MeOH/CH$_2$Cl$_2$) to yield 32.5 mg (37%) of **12** as a pale yellow solid: ^{1}H NMR (400 MHz, CD$_3$OD, δ) 1.44 (s, CH$_3$, 18H), 2.40–2.54 (m, CH$_3$, 6H), 2.61–3.65 (m, CH$_2$, 25H), 3.73–3.81 (m, CH$_2$, 2H), 4.01–4.16 (m, CH$_2$, CH, 3H), 4.22–4.31 (m, CH, 1H), 4.35–4.45 (m, CH, 1H), 4.69 (brs, CH, 1H), 5.81 (d, J = 8.0 Hz, CH, 1H), 6.06 (d, J = 8.0 Hz, CH, 1H), 6.38 (s, CH, 1H), 7.33–7.41 (m, CH, 1H), 7.84 (d, J = 4.0 Hz, CH, 1H), 8.10 (d, J = 8.0 Hz, CH, 1H); ^{13}C NMR (101 MHz, CD$_3$OD, δ) 11.7 (CH$_3$), 16.1 (CH$_3$), 17.6 (CH$_3$), 27.2 (CH$_3$), 42.6 (CH$_2$), 42.8 (CH$_2$), 43.3 (CH$_2$), 43.4 (CH$_2$), 55.7 (CH$_2$), 55.8 (CH), 61.7 (CH$_2$), 61.8 (CH$_2$), 71.0 (CH), 71.1 (CH), 81.5, 87.6 (CH), 101.1 (CH), 114.9 (CH), 117.1 (CH), 117.2 (CH), 120.4, 127.9 (CH), 132.9, 139.7 (CH), 141.9, 142.0, 151.3, 151.4, 151.8, 153.2, 160.9, 162.7, 165.8, 165.9, 171.0; TLC: R_f = 0.5 (10% MeOH/CH$_2$Cl$_2$); LR-ESI-MS (m/z) [M+H]$^+$ calcd for $C_{46}H_{68}N_8O_{13}$, 941.5; found, 941.4.

14: Monoalkylated cyclen **13** (171 mg, 0.399 mmol) was dissolved in CH$_3$CN (4 mL). Na$_2$CO$_3$ (254 mg, 2.39 mmol) was added, followed by *tert*-butyl bromoacetate (257 mg, 194 μL, 1.32 mmol, 3.3 equiv.). The reaction mixture was heated at 70 °C for 16 h. The reaction mixture was filtered, the filtrate was concentrated, and the residue was purified by column chromatography [silica, CH$_2$Cl$_2$:MeOH (2 → 10%)] yielding a white solid (282 mg, 92%): ^{1}H NMR (400 MHz, CDCl$_3$, δ) 1.10–1.17 (m, 3H), 1.35–1.59 (m, 27H), 2.12–3.65 (m, 31H), 4.08 (br, 1H), 6.26 (s, 1H), 6.91 (br s, 1H), 7.66 (s, 1H); ^{13}C NMR (100 MHz, CDCl$_3$) δ 12.4, 17.4, 18.3, 18.9, 27.8, 28.0, 43.8, 48.3 (br), 50.0, 52.4 (br), 55.4, 55.5, 55.6, 57.6, 81.6, 81.7, 81.8, 115.5, 116.6, 120.3, 127.9, 131.7, 142.1, 151.9, 152.1, 160.2, 170.2, 172.7; ESI-MS obsd 793.6, calcd 794.4 [(M+Na)$^+$, M = $C_{41}H_{65}N_5O_9$]; HR-ESI-MS obsd 794.4678, calcd 794.4674 (M+Na)$^+$.

15: The *tris*-tert-butyl ester-protected ligand (**14**, 66 mg, 0.068 mmol) was dissolved in CH$_2$Cl$_2$ (6 mL). TFA (6 mL) was added, and the reaction mixture was stirred at room temperature for 19 h. The reaction mixture was concentrated, and the solid residue was dried under vacuum yielding a brown solid (82 mg, TFA-salt): ^{1}H NMR (300 MHz, CD$_3$OD) δ 2.46 (s, 3H), 3.20–4.13 (m, 24H), 6.45 (s, 1H), 7.32, 7.64 (ABq, J = 8.7 Hz, 2H), 7.76 (s, 1H); ^{13}C NMR (75 MHz, CD$_3$OD, multiple conformers, all peaks reported) δ 17.7, 50.8–54.1 (m), 105.2, 110.7, 114.6, 114.9, 116.7, 117.2, 118.4, 122.3, 124.9, 138.2, 140.7, 150.4, 164.1 (+TFA: 161.1, q).

Alternative purification: The crude product was dried overnight under vacuum. The residue was dissolved in a small amount of CH$_3$CN:H$_2$O (3:1), and the sample was loaded onto a silica chromatography column (packed with CH$_3$CN). Elution [CH$_3$CN:H$_2$O (3:1 → 2:1), then CH$_3$CN:H$_2$O:NH$_3$ (aq.) (3:1:1)] afforded a yellow film that was dissolved in a minimum amount of water, filtered through a plug of cotton wool, and the solution was freeze-dried giving a yellow foam (65.5 mg, 48% from monoalkylated cyclen **13**): ^{1}H NMR (400 MHz, D$_2$O) δ 1.97–2.08 (br s, 4H), 2.24–4.25 (~24H), 5.98 (br, 1H), 6.74 (br, 1H), total 2H: 7.16–7.17 (ad,

J = 8.0 Hz), 7.28 (br); ^{13}C NMR (100.6 MHz, D$_2$O, multiple conformers, all peaks reported) δ 17.9, 18.1, 48.1, 56.7, 57.6, 58.7, 59.0, 63.9, 105.8, 115.1, 116.9, 117.2, 125.0, 137.1, 138.6, 150.7, 163.8, 170.0, 173.2, 179.8, 179.9; RP-HPLC t_R = 4.371 min; ESI-MS obsd 599.4, calcd 599.2 [(M+K)$^+$, M = C$_{26}$H$_{36}$N$_6$O$_8$], obsd 621.2, calcd 621.2 (M+K+Na−H)$^+$, obsd 643.4, calcd 643.2 (M+K+2Na−2H)$^+$, obsd 579.3, calcd 579.2 (M+K−2H)$^-$; HR-ESI-MS obsd 583.24889, calcd 583.24868 (M+Na)$^+$.

L^{4}Ln: A mixture of the triacid **15** (∼0.01 mmol) and the appropriate LnCl$_3$ (∼2–3 equiv) were dissolved in MeOH (100 µL), with a few drops of water added to facilitate dissolution. The reaction mixture was stirred at 45 °C for 48–72 h, and the progress of the reaction was monitored by RP-HPLC. After complete consumption of the ligands, the reaction mixture was concentrated to dryness. The residue was dissolved in a small amount (100–200 µL) of H$_2$O or MeOH, and the solution was added to a large volume (1–2 mL) of acetone or Et$_2$O, respectively. The precipitate was collected by centrifugation. The solid residue was dissolved in a minimum amount of water, and the solution was freeze-dried giving a yellow-gray foam. **L^{4}Eu**: RP-HPLC 8.645, 9.113 (sh) min; HR-ESI-MS obsd 754.19669, calcd 754.19573 [(M+H)$^+$, M = C$_{29}$H$_{38}$N$_5$O$_9$Eu]; λ_{em} = 384, 579, 588, 593.5, 614.5, 653, 687.5, 700 (679–707) nm (λ_{ex} = 320 nm). **L^{4}Tb**: RP-HPLC 8.564, 9.092 (sh) min; HR-ESI-MS obsd 760.20281, calcd 760.19957 [(M+H)$^+$, M = C$_{29}$H$_{38}$N$_5$O$_9$Tb]; λ_{em} = 384, 487, 541.5, 546, 587.5, 620 nm (λ_{ex} = 320 nm).

General procedure for **18** and **19**: Compound **17** (160 mg, 0.312 mmol, 1 equiv), Cs$_2$CO$_3$ (233 mg, 0.718 mmol, 2.3 equiv), and KI (104 mg, 0.625 mmol, 2 equiv) were suspended in acetonitrile (15 mL). Compound **8** or **16** (100 mg, 0.312 mmol, 1 equiv) was dissolved in DMF (2.5 mL) and was added to the suspension of **17**. The reaction mixture was heated at 55 °C for 48 h under Ar. The reaction mixture was filtered and the filtrate was dried under reduced pressure. Resulting oil was purified with silica gel (neutralized by pre-treating with a mixture of NH$_4$OH/MeOH/CH$_2$Cl$_2$ (0.5:3:8)) column chromatography (2.5 → 5% MeOH/CH$_2$Cl$_2$) to yield 80 mg (32%) of **18** or **19** as a yellow solid. For **18**: ^{1}H NMR (400 MHz, CDCl$_3$, δ) 1.20–1.58 (m, CH$_3$, 27H), 1.86–3.60 (m, CH$_2$, 24H), 3.74–3.91 (m, CH$_2$, 2H), 3.45 (solvent peak, MeOH), 4.27 (s, CH, 1H), 4.49 (brs, CH, 1H), 4.79 (brs, CH, 1H), 5.65 (brs, CH, 1H), 6.12 (d, J = 8.0 Hz, CH, 1H), 7.48–7.60 (m, CH, 1H), 7.89 (brs, NH, 1H); ^{13}C NMR (101 MHz, CDCl$_3$, δ) 27.9 (CH$_3$), 50.6 (CH$_2$), 50.7 (CH$_2$), 55.6 (CH$_2$), 55.8 (CH), 56.6 (CH$_2$), 62.4 (CH$_2$), 82.6, 102.4 (CH), 150.7, 172.3; TLC R_f = 0.4 (10% MeOH/CH$_2$Cl$_2$); LR-ESI-MS (m/z) [M+H]$^+$ calcd for C$_{37}$H$_{63}$N$_7$O$_{12}$, 798.5; found, 798.3; HR-ESI-MS obsd 820.4418, calcd 820.4427 [(M+Na)$^+$, M = C$_{37}$H$_{63}$N$_7$O$_{12}$]. For **19**: ^{1}H NMR (400 MHz, CD$_3$OD, δ) 1.40–1.60 (m, CH$_3$, 27H), 1.94–3.77 (m, CH$_2$, 28H), 3.95–4.07 (m, CH$_2$, 2H), 4.20–4.24 (m, CH, 1H), 5.69–5.79 (m, CH, 2H), 7.68 (d, J = 8.0 Hz, CH, 1H); ^{13}C NMR (101 MHz, CD$_3$OD, δ) 27.1 (CH$_3$), 55.4 (CH$_2$), 55.5 (CH$_2$), 71.0 (CH), 71.1 (CH), 81.3, 81.4, 82.1 (CH), 101.6 (CH), 141.8 (CH), 150.7, 164.5, 172.4, 173; TLC R_f = 0.42 (10% MeOH/CH$_2$Cl$_2$); LR-ESI-MS (m/z) [M+H]$^+$ calcd for C$_{37}$H$_{63}$N$_7$O$_{12}$, 798.5; found, 798.3.

2.2. General procedure for L^{3}Ln, L^{5}Ln, and L^{6}Ln

2.2.1. Deprotection of ligand

Compound **12**, **18**, or **19** (0.05 mmol) was dissolved in TFA/CH$_2$Cl$_2$ (1:1, 2 mL) and the solution was stirred at ambient temperature for 24 h. To the reaction mixture toluene (1 mL) was added and the solvents were removed under reduced pressure. The resulting residue was triturated with toluene/acetonitrile (×3, 1:2, 2 mL) and with acetonitrile (×2, 2 mL) to obtain quantitative amounts of the deprotected ligand as a yellow glass-like solid which was used for next step without further purification. Deprotected **12**: ^{1}H NMR (400 MHz, D$_2$O, δ) 0.95 (brs, CH$_3$, 3H), 2.18 (s, CH$_3$, 3H), 2.27 (s, CH$_3$, 3H), 2.52–4.48 (m, CH$_2$, CH, 31H), 5.76

(brs, CH, 2H), 6.18 (s, CH, 1H), 7.16 (s, CH, 1H), 7.60 (s, CH, 1H), 7.70 (brs, CH, 1H); ^{13}C NMR (101 MHz, CD$_3$OD, δ) 11.7 (CH$_3$), 16.0 (CH$_3$), 17.3 (CH$_3$), 42.7 (CH$_2$), 43.3 (CH$_2$), 52.7 (CH$_2$), 54.7 (CH$_2$), 55.9 (CH$_2$), 56.0 (CH), 61.6 (CH$_2$), 61.7 (CH$_2$), 70.6 (CH$_2$), 70.7 (CH$_2$), 87.3 (CH), 87.5 (CH), 101.4 (CH), 114.7 (CH), 114.9 (CH), 116.8 (CH), 117.2 (CH), 117.6 (CH), 120.4, 127.7 (CH), 132.9, 139.4 (CH), 141.9, 151.3, 151.9, 153.2, 160.3, 160.6, 160.9, 162.8, 164.9, 165.9; LR-ESI-MS (m/z) [M+H]$^+$ calcd for C$_{38}$H$_{52}$N$_8$O$_{13}$, 829.4; found, 829.4. Deprotected **18**: ^{1}H NMR (400 MHz, D$_2$O/CD$_3$OD (12:1), δ) 2.83–3.86 (m, CH$_2$, 26H), 4.03–4.08 (m, CH, 1H), 4.20–4.25 (m, CH, 1H), 4.36–4.42 (m, CH, 1H), 5.77 (d, J = 8.0 Hz, CH, 1H), 5.88 (d, J = 8.0 Hz, CH, 1H), 7.75 (d, J = 8.0 Hz, CH, 1H); ^{13}C NMR (101 MHz, CDCl$_3$, δ) 55.6 (CH$_2$), 61.2 (CH$_2$), 70.0 (CH$_2$), 86.3 (CH), 87.1 (CH), 102.6 (CH), 114.8 (CH), 117.7 (CH), 141.2, 150.6, 165.7. Deprotected **19**: LR-ESI-MS (m/z) [M+H]$^+$ calcd for C$_{25}$H$_{39}$N$_7$O$_{12}$, 630.3; found, 630.1.

2.2.2. Metallation

Deprotected ligand (0.05 mmol, 1 equiv) was dissolved in MeOH (1 mL) and the pH of the solution was adjusted to 7–8. The required LnCl$_3$ salt (0.075 mmol, 1.5 equiv) was added and the reaction mixture was heated at 50 °C for 30 h while maintaining the pH at 6–7. The reaction mixture was centrifuged and the precipitate was separated. The supernatant was dried under reduced pressure and the resulting residue was dissolved in water (2 mL). The pH of the solution was raised to pH 12 by adding conc. NH$_4$OH, the solution was allowed to stand for 20 min at ambient temperature, and centrifuged. The liquid was separated and freeze dried. The resulting solid residue was dissolved in MeOH (1 mL) and precipitated with diethylether (4 mL) to yield the metal complex as an off-white solid. **L^{3}Eu**: LR-ESI-MS (m/z) M$^+$ calcd 979.3; found, 979.3; HR-ESI-MS obsd 979.2711, calcd 979.2709 [M$^+$, M = C$_{38}$H$_{50}$N$_8$O$_{13}$Eu]; **L^{3}Tb**: LR-ESI-MS (m/z) M$^+$ calcd 985.3; found, 985.3; HR-ESI-MS obsd 985.2713, calcd 985.2745 [M$^+$, M = C$_{38}$H$_{50}$N$_8$O$_{13}$Tb]; **L^{5}Nd**: LR-ESI-MS (m/z) (M+H)$^+$ calcd 769.2; found, 770.1; HR-ESI-MS obsd 791.1385, calcd 791.1391 [(M+Na)$^+$, M = C$_{25}$H$_{36}$N$_7$O$_{12}$Nd]; **L^{6}Nd**: LR-ESI-MS (m/z) (M+H)$^+$ calcd for C$_{25}$H$_{36}$N$_7$O$_{12}$Nd, 769.2; found, 769.1; HR-ESI-MS obsd 769.1539, calcd 769.1572 [(M+H)$^+$, M = C$_{25}$H$_{36}$N$_7$O$_{12}$Nd].

2.2.3. Sample preparation for UV–vis and luminescence measurements

Metal complexes were dissolved in water or deuterated water to obtain 0.5 mM solutions for luminescence studies. For UV–vis measurements solutions of 0.16 mM (for **L^{3}Ln**) or 0.02 mM (for **L^{1}Ln** and **L^{2}Ln**) were used, respectively. The absorption spectra shown in Fig. 1 were recorded in HEPES (0.1 M, pH 7).
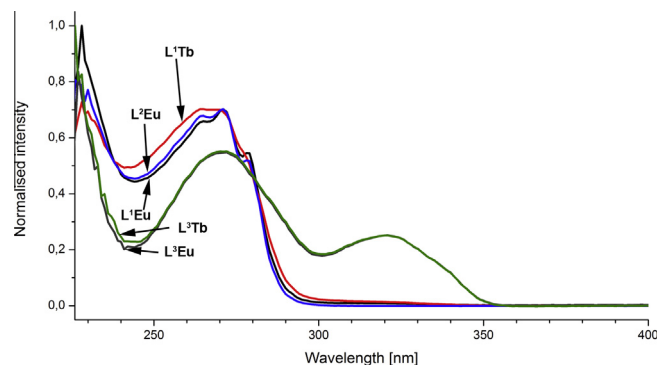

Fig. 1. Absorption spectra of Eu(III)- and Tb(III)-complexes in HEPES (0.1 M) solution at pH = 7 (black: **L^{1}Eu**, red: **L^{1}Tb**, blue: **L^{2}Eu**, gray: **L^{3}Eu**, green: **L^{3}Tb**). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

3. Results and discussion

Two types of nucleotide-appended Ln(III)-complexes were prepared. The first type was based on the well-established and versatile dipicolinic acid (dpa) Ln(III)-binding site [39–48]. The functionalized pyridine is both the chelator and the antenna. This simple framework would enable us to compare the photophysical properties of our complexes to existing standards, specifically, to evaluate the effect of the nucleotide on the complex photophysics. The second complex type was built around a cyclen (1,4,7,10-tetraazacyclododecane) framework, which affords tight metal ion binding, along with up to four secondary nitrogen atoms available for functionalization. The cyclen-based complexes were modeled on known compounds carrying a coumarin 2-derived antenna and a nucleotide in the 1N- and 4N-positions [37,49]. Depending on the nucleotide, the 1,4-functionalized complexes were poorly emissive. Here we chose uridine as it was an inefficient quencher. To maximize their separation and further minimize quenching, the antenna and the uridine were attached to the 1N- and 7N-positions.

The synthesis of the dpa-complexes is shown in Schemes 1 and 2. Briefly, dpa-monomethyl ester **1-Me** was reacted with 2′- or 5′-aminouridine with EDCI/6-Cl-HOBt as coupling reagent. The solubility of 5′-aminouridine in organic solvents was too low for an efficient reaction, therefore the coupling was conducted in a water-THF mixture. The relatively low yield of this reaction could be explained by competing diimide hydration which deactivates

the EDCI. Ester hydrolysis under basic conditions afforded the uridine-modified dpa-derivatives **4** and **6** in excellent yield. Mixing these ligands with pre-formed Ln(III) bis-dpa monosodium salts afforded 1:1:2-complexes (Ln:**4**/**6**:dpa) **L¹Eu**, **L¹Tb** and **L²Eu** in good to excellent yields. HPLC-analysis of **L¹Eu** showed the presence of a major and a minor species. One component was observed for **L²Tb** under the same conditions, however, the photophysical properties are also consistent with the presence of multiple species (*vide infra*).

The cyclen-based complexes were designed to contain the octadentate dota Ln-binding site. This in turn meant that the antenna and the nucleotide were incorporated by alkylation of the secondary nitrogens with the appropriate chloroacetamides. The required dialkylated cyclen starting material (**9** in Scheme 3) is readily available on a multigram scale from cyclen in three steps [35,36,50].

The 2-aminouridine chloroacetamide **8** was prepared by the reaction of **2a** with chloroacetic anhydride in MeOH with DMAP as both the base and catalyst (Schemes 3 and 4). Treatment of **9** with **8** in the presence of NaHCO₃ yielded the monofunctionalized product **10** in good yield after chromatography on silica gel. The bicarbonate base was chosen as its use has been shown to reduce alkylation of both secondary nitrogens [51,52]. The alkylation of the remaining secondary nitrogen was more difficult, affording the protected ligand **12** in 37% yield. The lower yield of this step could be due to the steric congestion around the nitrogen. *Tert*-butyl ester cleavage with TFA, followed by complexation with Eu
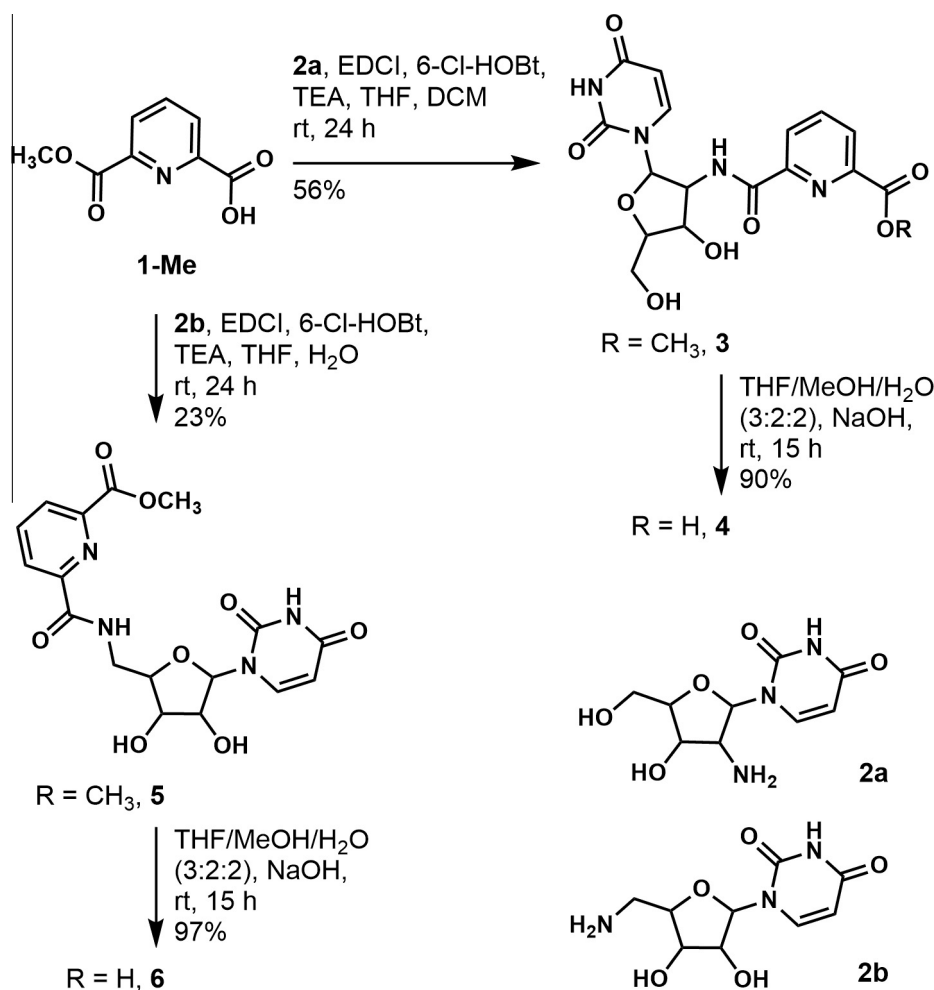
Scheme 1. Synthesis of uridine-appended dpa-derivatives **4** and **6**.
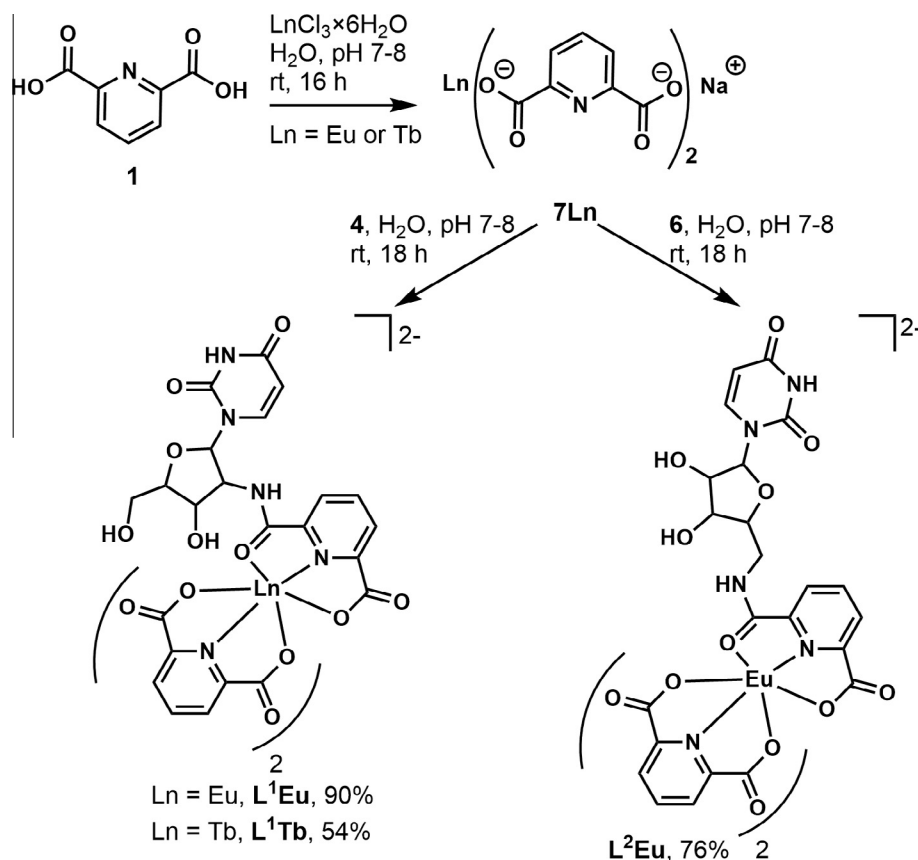
Scheme 2. Eu- and Tb-tris-dipicolinate complexes **L¹Eu**, **L¹Tb** and **L²Eu** with attached uridine.

(III) or Tb(III) afforded the final complexes after freeze-drying from water. Reference complexes **L⁴Eu** and **L⁴Tb** lacking the uridine were also prepared (Scheme 3).

The potential energy transfer acceptor Nd(III)-complexes were based on the same dota binding site. Trialkylated cyclen **17** was reacted with uridine chloroacetamide derivatives **8** or **16** in the presence of Cs_2CO_3 and KI to afford the protected ligands **18** or **19** carrying a 2′-linked or a 5′-linked uridine, respectively. Acidic treatment and exposure to $NdCl_3$ yielded **L⁵Nd** and **L⁶Nd** in excellent overall yield.

All new diamagnetic compounds were fully characterized by ¹H and ¹³C NMR spectroscopy and high resolution mass spectrometry. The paramagnetic Ln(III)-complexes were characterized by high resolution mass spectrometry, HPLC, and absorption and emission spectroscopy. For **L¹Tb** and **12** we were unable to obtain high resolution MS data, but low resolution MS-data were available, and all other characterization data were in line with the expected structures. Furthermore, we expect Eu(III)- and Tb(III)-complexes of the same ligands to behave similarly due to the similar reactivity of the metal ions. For **12**, both precursors and downstream products were fully characterized.

4. Photophysical characterization

The UV–vis absorption, and steady-state and time-resolved emission spectra of the Ln(III)-complexes were recorded in water (Figs. 1 and 2, Table 1). The absorption spectra of **L¹Eu** and **L¹Tb** had an absorption band typical of the dpa chromophore centered around 275 nm with a cutoff at ∼290 nm [45]. The Ln(III)-excitation spectra for the Eu(III)- and Tb(III)-complexes showed a single intense band in the same region as expected for ligand-sensitized

Ln(III)-emission. The ligands containing the coumarin 2-antenna were excitable at longer wavelengths, up to ∼350 nm, antenna excitation resulted in typical Ln(III)-emission in all cases. The luminescence quantum yields for the dpa-type complexes (**L¹Ln**, **L²Ln**) were ∼10-fold lower than those of non-modified $Cs_3Ln(dpa)_3$-species (Table 1), which have Φ(Eu) = 29 ± 2% and Φ(Tb) = 21 ± 1% [43]. Similarly, **L³Eu** and **L³Tb** were much less emissive than reference complexes **L⁴Eu** and **L⁴Tb**. A possible explanation for the diminished quantum yields could be photoinduced electron transfer between the antennae and the uridine [53]. An alternative explanation would be photoredox quenching of Ln(III) via the formation of Ln(II) by electron transfer from an excited antenna/uridine. However, electron transfer is unlikely to Tb(III), as its +2-oxidation state is difficult to access, even though this process is known to operate for Eu(III) [54,55]. Therefore, a Ln(III)-independent quenching processes is likely.

The Eu(III)- and Tb(III)-emitters were investigated with time-resolved emission spectroscopy. The luminescent lifetimes of **L¹Ln–L⁴Ln** are listed in Table 1. Satisfactory fitting was possible with monoexponential decay in all cases. Regioisomeric **L¹Eu** and **L²Eu** have comparable lifetimes, suggesting that the difference between the Eu(III)-environments and the resulting complex photophysics is small. This implies that placing these complexes in the 3′- or 5′-termini of oligonucleotide should yield conjugates with similar photophysical properties. **L¹Tb** had a lifetime of 1.24 ms in water. These Ln(III) emission lifetimes are shorter than those of non-functionalized tris-dpa-complexes (τ_{obs} = 1.7 ± 0.1 ms for $Cs_3Eu(dpa)_3$ and τ_{obs} = 1.74 ± 0.01 ms for $Cs_3Tb(dpa)_3$) [43]. The number of Ln(III)-coordinated water molecules (q) were determined using the Horrocks method [56] from the luminescent lifetimes in H_2O and D_2O.
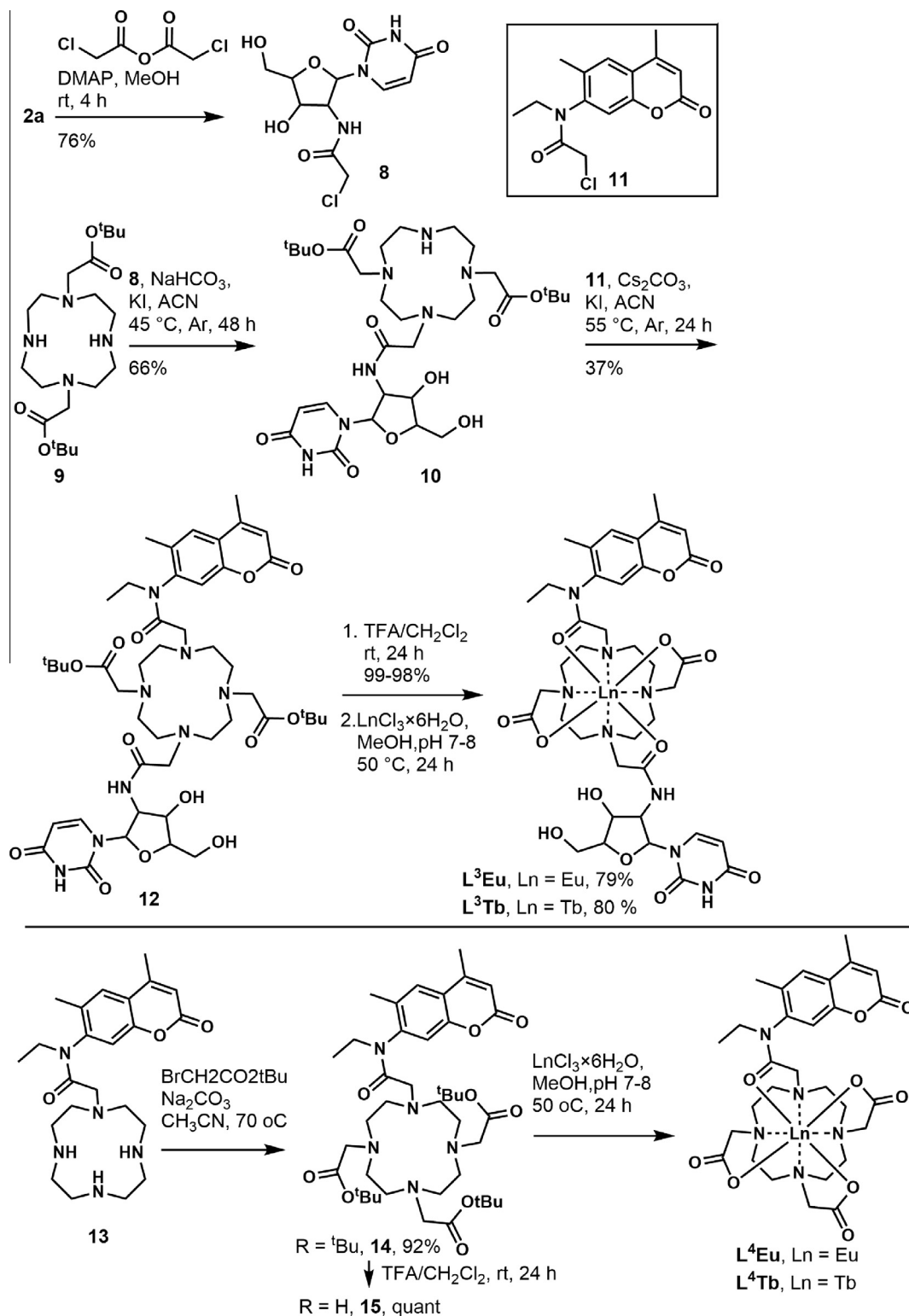
Scheme 3. Synthesis of Eu(III)- and Tb(III)-complexes with coumarin 2-sensitizers and 2′-linked uridine appended onto a cyclen framework (top), and **L⁴Ln** reference compounds (bottom).

The luminescence decays had good monoexponential fits in all cases. However, biexponential fitting of the decays gave slightly better results for **L¹Eu**, **L²Eu** and **L³Tb**. Such observations need to be interpreted with caution, and several reasons are conceivable. An equilibrium between complexes with $q = 0$ and $q = 1$ could explain the non-monoexponential decay. The nonzero q values

are consistent with such a scenario. The increased q clearly contributes to the reduced Ln(III)-lifetimes and Ln(III)-quantum yields compared to Eu(dpa)$_3$ and Tb(dpa)$_3$ (which are $q = 0$), and can be caused by weaker coordination of the amide donor compared to the carboxylate, or increased steric hindrance and consequent opening up of the complex due to the aminouridine substitutent.
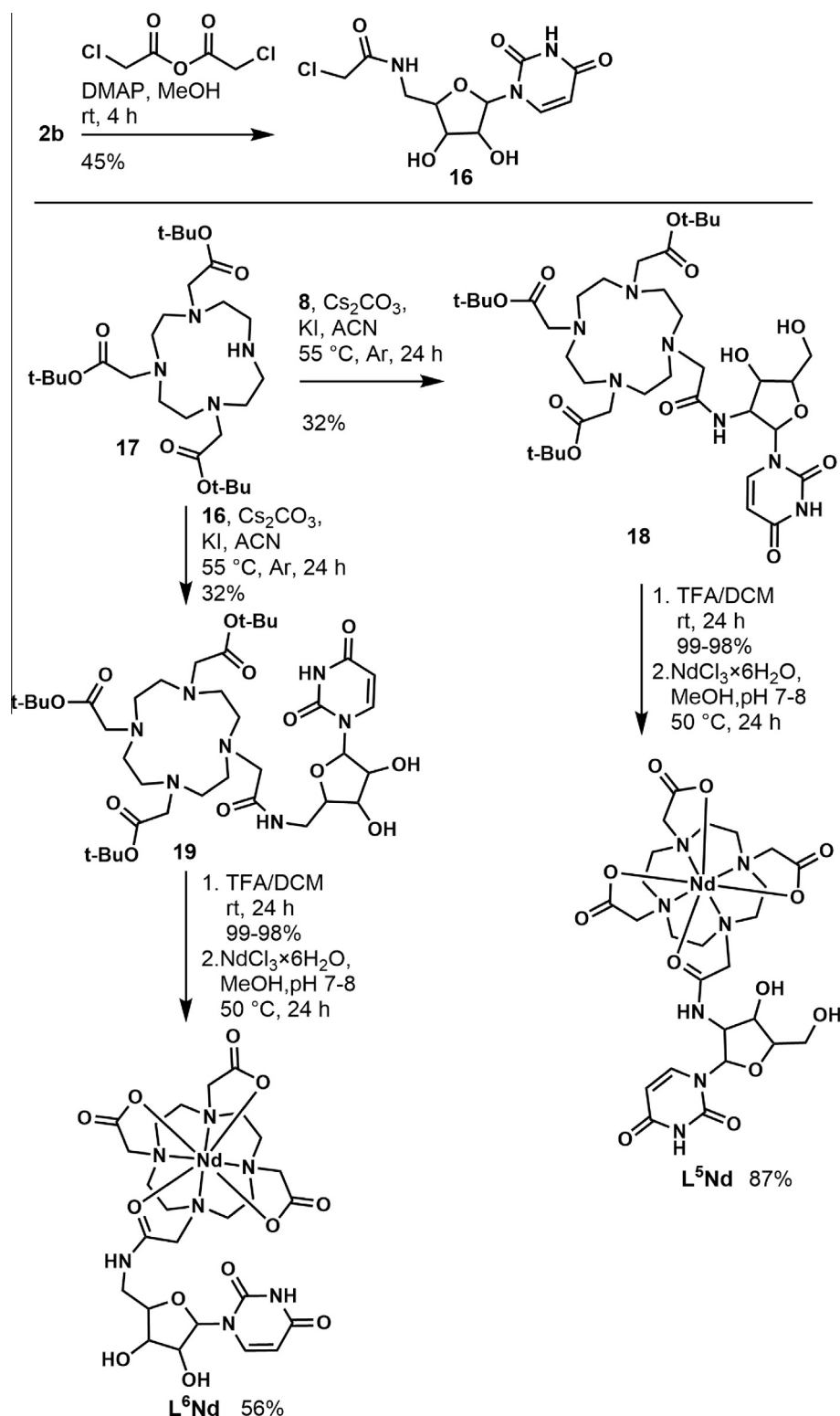
Scheme 4. Synthesis of Nd(III)-complexes.

Ligand exchange to form various Ln(dpa)$_n$(**6**)$_{3-n}$ or Ln(dpa)$_n$(**4**)$_{3-n}$ species *in situ* may be possible. Furthermore, as the uridine moiety is a single enantiomer, and both the dpa- and the cyclen-frameworks can form chiral complexes [57], the presence of multiple diastereomers is conceivable (Fig. 3). We investigated the presence of diastereomers by NMR spectroscopy. We prepared the diamagnetic homoleptic 1:3 (Lu(III): **4**) species, where exchange between

dpa and **4** is not possible. The ^{1}H NMR spectrum of the crude product was complex, showing multiple components (Fig. S13). We also recorded the ^{1}H NMR spectrum of **L^{1}Eu**. Signals were broadened and paramagnetically shifted with peaks up to 12.5 ppm, as expected for a Eu(III)-complex (Fig. S14). The broadness of the signals precluded their unambiguous assignment. Therefore, we cannot definitively say whether we have diastereomeric complexes in
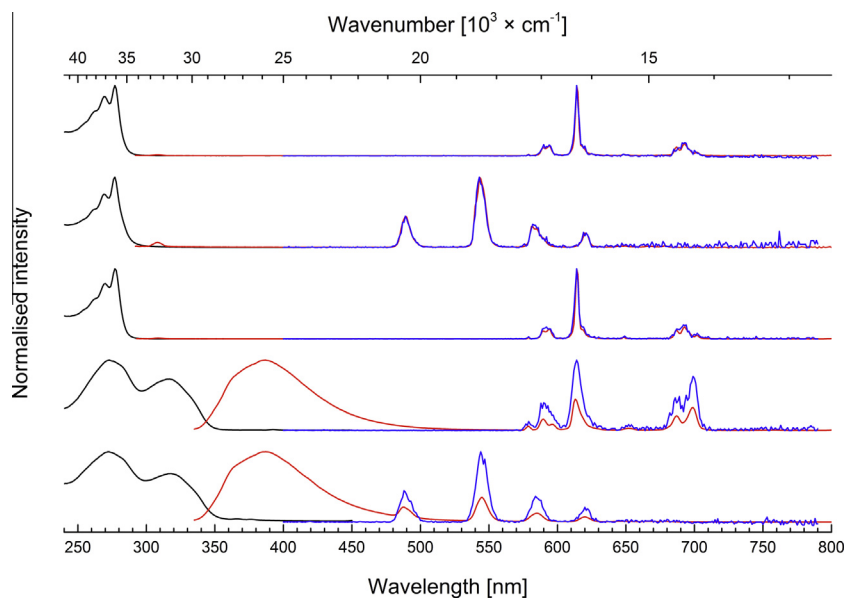
Fig. 2. Steady-state excitation (black, λ_{em} = 614 nm (Eu), 543 nm (Tb)) and emission (red, λ_{ex} = 278 nm (**L^{1}Eu** (1st from top), **L^{1}Tb** (2nd), **L^{2}Eu** (3rd)), 320 nm for **L^{3}Eu** (4th), **L^{3}Tb** (5th)) and time-resolved emission spectra (blue, λ_{ex} as above, initial delay: 0.05 ms, sample window: 0.2 ms) of Eu- and Tb-complexes. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Table 1
Luminescent lifetimes and water coordination numbers of **L^{1}Ln**, **L^{2}Eu**, and **L^{3}Ln**.

	$\tau(H_2O)$ (ms)	$\tau(D_2O)$ (ms)	q^c	$\Phi_{Ligand}{}^d$	$\Phi_{Ln}{}^d$
L^{1}Eu	1.12 ± 0.003[a,e]	3.46 ± 0.01	0.63 ± 0.001	0%	3.23%
	0.30 ± 0.05 (48%), 1.49 ± 0.021 (52%)[b]				
L^{1}Tb	1.24 ± 0.02[a]	1.72 ± 0.03	0.95 ± 0.02	0%	1.33%
L^{2}Eu	0.94[a,e]	3.40	0.80	0%	3.22%
	1.58 ± 0.02 (52%), 0.33 ± 0.004 (48%)[b]				
L^{3}Eu	0.42 ± 0.007[a]	1.73 ± 0.06	1.89 ± 0.02	1.0%	0.14%
L^{3}Tb	0.68 ± 0.03[a,e]	2.05 ± 0.01	3.32 ± 0.56	0.97%	0.10%
	0.18 ± 0.003 (53%), 1.14 ± 0.009 (47%)[b]				
L^{4}Eu	0.65 ms	n.d.	n.d.	0.63%	1.89%
L^{4}Tb	0.515 ms	n.d.	n.d.	0.64%	2.23%

[a] Monoexponential fit.
[b] Biexponential fit.
[c] Determined using $q = A_{Ln}[(1/\tau(H_2O) - 1/\tau(D_2O)]$, where A_{Eu} = 1.05 and A_{Tb} = 4.2 ms.
[d] Determined using the optically dilute method with quinine sulfate in 0.05 M H_2SO_4 as the reference.
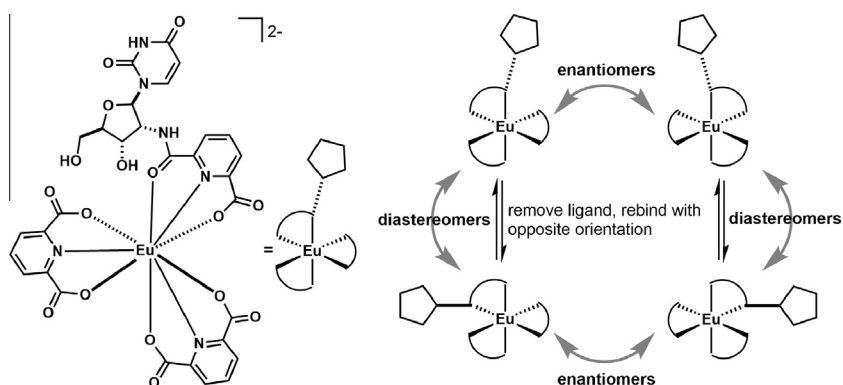[e] Biexponential fit of better quality based on R^2 and χ^2.

Fig. 3. Possible stereoisomers of Ln(dpa)$_2$(**4**) species in solution.

either of these samples. The cyclen-based complexes also had larger than expected q values. The q = 3.32 for **L^{3}Tb** is unrealistic, as the q of **L^{3}Tb** and **L^{3}Eu** should not differ drastically. This indicates additional Tb(III) quenching pathways in **L^{3}Tb**, e.g. by energy back transfer to the antenna triplet from the Tb(III).

Finally, we investigated the possibility of energy transfer between **L^{3}Eu** and **L^{5}Nd** (Fig. 4). As both complexes were equipped with only a single uridine, hydrogen bonding in aqueous solution was not expected to be efficient. Therefore, we conducted the experiment in a less competitive water: acetonitrile (45:255)
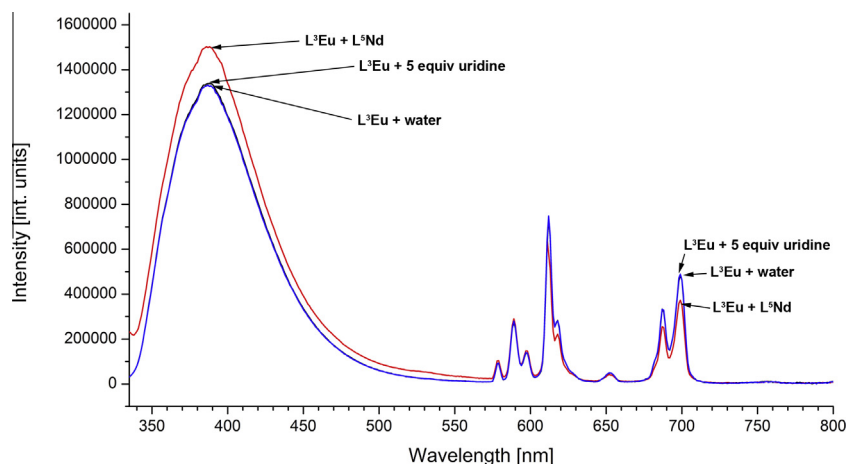
Fig. 4. Emission spectra of **L³Eu** in the presence of 5 equiv. **L⁵Nd** in H₂O: acetonitrile (45:255), blue: **L³Eu** + water, red: **L³Eu** + **L⁵Nd**, black: **L³Eu** + 5 equiv. uridine. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

mixture. Addition of a stock solution of **L⁵Nd** to **L³Eu** resulted in an increased residual antenna emission and a decrease in the Eu(III)-luminescence. The addition of only uridine did not affect either the antenna fluorescence or the Eu(III)-emission. The Eu(III)-lifetime ($\tau = 0.335 \pm 0.002$ ms) decreased in the presence of **L⁵Nd** ($\tau = 0.22 \pm 0.01$ ms (monoexp.) or $\tau_1 = 0.284 \pm 0.015$ ms, $\tau_2 = 0.132 \pm 0.014$ ms (biexp.)), and remained unaffected when only uridine ($\tau = 0.33 \pm 0.02$ ms) was added. Eu-to-Nd energy transfer has been proposed previously as an explanation for shortened Eu-lifetimes in dinuclear Ln₂-complexes consisting of dota-bound Eu(III) and Nd(III) connected via aryl groups [58]. The instrumentation available to us was not sensitive enough to detect Nd-emission, which is expected to be very dim even under optimal circumstances, given the low intrinsic quantum yield of Nd(III) and the presence of coordinated water molecules around both Nd(III) and Eu(III). Therefore, we were not able to obtain direct evidence for Eu-to-Nd energy transfer. However, interlanthanide energy transfer is in line with our observations. The increased antenna fluorescence observed upon **L⁵Nd**-addition is not readily explained, and is currently being investigated.

5. Conclusions

Luminescent Eu(III)- and Tb(III)-complexes carrying coumarin or dpa sensitizing antennae in two chelating frameworks were synthesized and characterized. The complexes were attached to either 2′- or 5′-aminouridine. Antenna excitation resulted in long-lived Ln(III)-emission in all cases. The luminescence quantum yields decreased compared to analogous complexes lacking the nucleotide. The reported complexes are useful models of oligonucleotide-bound luminescent Ln(III)-probes, which are envisioned as emissive labels, tools for studying conformational changes, and sensitive reporters of the nucleic acid microenvironment.

Acknowledgments

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ica.2016.07.047.

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